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RESEARCH ARTICLE

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Expression of the prognostic marker IL-8 correlates with the immune signature and epithelial-mesenchymal transition in breast cancer

Huifeng Liao[1,2] | Huayan Li[3] | Jin Song[4] | Hongye Chen[2] | Huiyan Si[2] | Junhua Dong[4] | Jiandong Wang[2] | Xue Bai[1,2]

[1]The Second School of Clinical Medicine, Southern Medical University, Guangzhou, China

[2]Department of General Surgery, The First Medical Center of Chinese PLA General Hospital, Beijing, China

[3]Zhujiang Hospital, Southern Medical University, Guangzhou, China

[4]Department of General Surgery, The Seventh Medical Center of Chinese PLA General Hospital, Beijing, China

Correspondence
Jiandong Wang and Xue Bai, Department of General Surgery, The First Medical Center of Chinese PLA General Hospital, Beijing, 100853, China.
Email: vicky1968@163.com and drbaixue@126.com

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Abstract

Background: IL-8 has been implicated in the malignant progression of various types of cancers; however, the precise molecular mechanisms associated with IL-8 in breast cancer (BRCA) are unclear.

Methods: We analyzed the clinical signature and immune characteristics of BRCA patients and its correlation with IL-8 expression using The Cancer Genome Atlas (TCGA) datasets. The role of IL-8 in epithelial-mesenchymal transition (EMT) was verified through Western blotting, Cell Counting Kit-8 assay, and wound healing assays, as well as cell invasion experiments.

Results: Through a comprehensive bioinformatics study, we determined that high IL-8 expression was associated with poor prognosis. Enrichment analysis revealed that high IL-8 expression was enriched in immune-related processes and cancer-related signaling pathways. In addition, IL-8 was associated with most of the immune-infiltrating cells, and high IL-8 expression indicated poor response to immunotherapy. Importantly, we found that IL-8 induced EMT in vitro.

Conclusions: Taken together, our data indicate that IL-8 may be a potential and valuable prognostic marker in BRCA, which may induce adverse outcomes by modulating the immune response and promoting EMT in BRCA patients.

KEYWORDS
breast cancer, epithelial-mesenchymal transition, immune infiltration, prognosis, tumor microenvironment

1 | INTRODUCTION

Breast cancer (BRCA) is one of the most common malignant tumors, seriously endangering women's physical and mental health. According to the latest data, the incidence of BRCA continues to rise, and has replaced lung cancer as the number one cancer worldwide.[1] Despite significant advances in the diagnosis and treatment of BRCA, including systemic treatment with chemotherapy, endocrine therapy, and targeted therapy, BRCA remains the leading cause of cancer-related death in women worldwide.

Therefore, there is an urgent need to unravel the molecular mechanism underlying BRCA tumorigenesis, which may enable the development of novel therapeutic strategies to improve patient survival.

IL-8, a member of the CXC chemokine family, functions as an important regulator in the tumor microenvironment (TME) by binding to the CXCR1 and CXCR2 receptors.[2] According to previously published studies, IL-8 is not only involved in processes such as inflammatory stimulation and wound healing, but also participates in regulating the immune response in various inflammatory diseases, and promotes tumorigenesis through multiple signal transduction pathways.[3–5] Secreted by neutrophils, fibroblasts, and tumor cells, IL-8 expression correlates with the biological characteristics of various tumors, including melanoma, non-small cell lung cancer, liver cancer, pancreatic cancer, colon cancer, and prostate cancer.[6–11] For example, higher IL-8 levels in the peripheral blood are correlated with higher stage, grade, and tumor burden in various types of cancers.[12–14] Zhai et al. demonstrated that IL-8 promoted platinum-based chemoresistance in gastric cancer by upregulating ABCB1 through the NF-κB signaling pathway.[15] Yuen et al. reported that elevated plasma IL-8 levels were predictive of the adverse outcomes during immune checkpoint blockade in metastatic urothelial carcinoma and metastatic renal cell carcinoma patients.[16] Moreover, relevant studies have revealed that IL-8 participated in tumor angiogenesis, inhibition of apoptosis and EMT.[17–19] In the TME, tumor cells and stromal cells recruited immunosuppressive cells by secreting inflammatory factors such as IL-4, IL-6, and IL-10, forming an immunosuppressive microenvironment leading to tumor progression.[20–23] However, the role of IL-8 in BRCA is still unclear. Systematic analysis of the biological significance of IL-8 and the molecular mechanism associated with IL-8 mediated tumorigenesis may enable the development of novel strategies for treating BRCA patients.

We aimed to explore the potential function of IL-8 in BRCA. Here, we investigated the relationship between IL-8 expression and tumor-infiltrating immune cells. Moreover, we showed that IL-8 promoted EMT in vitro. Our study revealed the role of IL-8 in modulating the immune response and EMT, providing evidence for further studies exploring IL-8-based therapeutics for treating BRCA.

2 | MATERIALS AND METHODS

2.1 | Data acquisition and processing

RNA-seq data and clinical data of 1226 BRCA patients (including 1113 tissue samples and 113 adjacent non-tumor tissue samples) were obtained from TCGA cohort. Based on the median level of IL-8 expression, we categorized the data into high and low IL-8 expression groups.

2.2 | Tumor immune single-cell hub database

The single-cell analysis of the IL-8 expression in the TME was investigated with reference to the tumor immune single-cell hub (TISCH) database.[24]

2.3 | PrognoScan database and Kaplan Meier Plotter database

Three cohorts of patients, namely, GSE7390 (n = 198), GSE1456-GPL96 (n = 159), and GSE 4922-GPL96 (n = 249) from the PrognoScan database[25] was applied to examine the prognostic value of IL-8. Kaplan Meier Plotter is an online database.[26] We selected the "auto select best cutoff" and "only JetSet best probe set" to analyze the relationship of the IL-8 expression with the overall survival (OS), distant metastasis-free survival (DMFS), progression-free survival (PFS), and post-progression survival (PPS).

2.4 | Enrichment analysis

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of IL-8 and its co-expressed genes were performed by R package "clusterProfiler", "enrichplot", and "ggplot2". Gene Set Enrichment Analysis (GSEA) software (v4.3.2) was used to further understand the molecular mechanisms of the IL-8 expression in BRCA.

2.5 | Correlation of IL-8 expression and immune signature in BRCA

The correlation of the IL-8 expression with the immune cell subpopulation was analyzed by using CIBERSORT.

2.6 | TIMER2.0 database

The correlation between the IL-8 expression and EMT-related genes was explored by the TIMER2.0 database.[27]

2.7 | Cell culture, wound healing, and invasion assays

MCF-7 and MDA-MB-231 cells were stored in our laboratory and cultured in DMEM media and L-15 containing 10% serum, respectively. For the wound healing assays, when the appropriate cell density was reached, the cells were scratched with the tip of a plastic straw. For the invasion assays, a 1:8 dilution of matrigel (Corning) was coated on the transwell membranes (Corning). While serum-free media was used to seed the cells in the upper chamber, medium

containing 10% serum was added to the bottom chamber. After 72-h incubation, the invaded cells were fixed in 4% paraformaldehyde and stained with crystal violet solution.

2.8 | Western blotting

The expression levels of Vimentin, GAPDH, N-Cadherin, and E-Cadherin proteins were detected by Western blotting, and all the primary and secondary antibodies were purchased from Cell Signaling Technology.

2.9 | Cytotoxicity assay

After 48-hour incubation with the chemotherapeutic drugs, Cell Counting Kit-8 (CCK-8) assay was conducted to measure the cell viability. 10 μl of fully mixed CCK-8 solution (Boster) was uniformly added to each well and the plates were incubated for 1 h. Using a microplate reader, the absorbance of the samples was measured at 450 nm for further analysis.

2.10 | Statistical analysis

Bioinformatical data was analyzed by the R 4.3.2 software, and the verification experiment analysis was performed using the SPSS 24.0 software. Quantitative data of the control group and the treatment group were compared by *t*-test after the normality test. The difference was considered to be statistically significant at $p < 0.05$.

3 | RESULTS

3.1 | Expression of IL-8 in the TME

The IL-8 expression in the TME was investigated using the TISCH web tool. Figure 1A shows the heatmap for the expression of IL-8 in various cell types in the five datasets. Based on the heatmap, it was obvious that IL-8 was mainly secreted by the neutrophils, monocytes/macrophages, and fibroblasts, and was relatively less expressed by the tumor cells (Figure 1B,C).

3.2 | Prognostic value of IL-8 expression

Data from TCGA implied that IL-8 was not related to prognosis and most of the clinical features (including age, gender, stage, and TMN) in BRCA (Figure 2A,B). However, GSE7390 ($n = 198$) and GSE1456-GPL96 ($n = 159$) from the PrognoScan database suggested that the IL-8 expression was significantly correlated with OS, DMFS, RFS, disease-specific survival (DSS), and disease-free survival (DFS) (Figure 2C). We further evaluated the prognostic potential of IL-8 in different BRCA subtypes using the Kaplan Meier Plotter database. The results revealed that, in almost all subtypes, high IL-8 expression was associated with poor prognosis, including OS, DMFS, RFS, and PPS (Figure 3A–D). Overall, IL-8 might be a potential prognostic marker for BRCA.

3.3 | Functional notes of IL-8

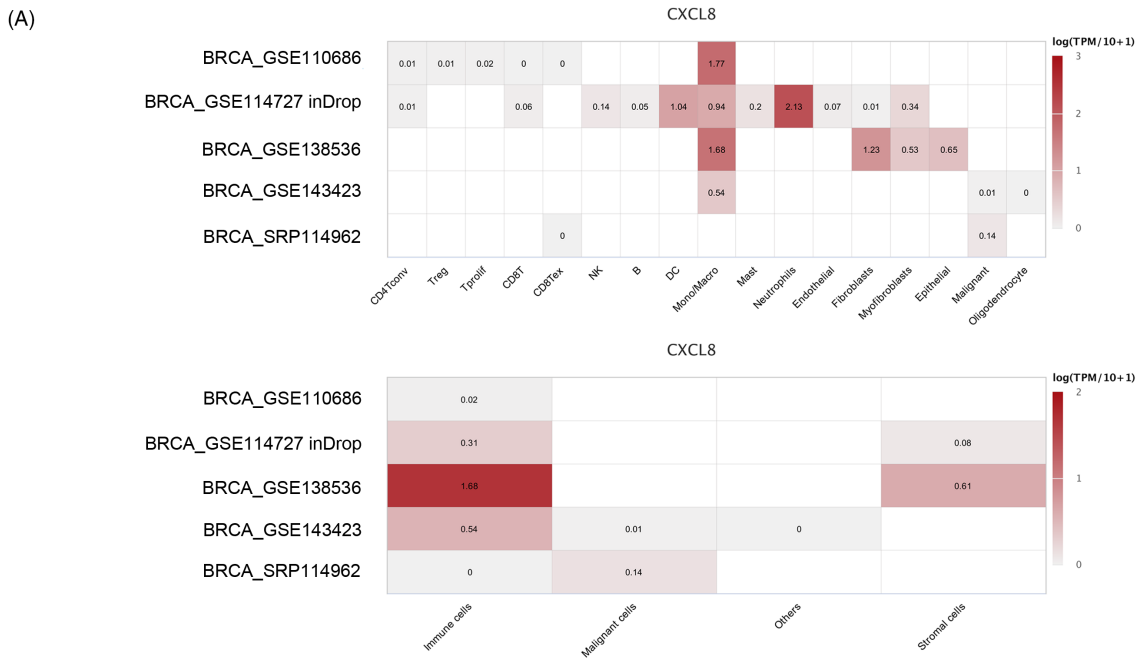
The heat map in Figure 4A shows the top 50 genes related to the high and low IL-8 expression groups. We next conducted the GO and KEGG analyses to better comprehend the potential biological function of IL-8. Figure 4B lists the top 10 important terms of BP, MF, and CC, implying that IL-8 may be involve inflammatory reactions and immune-related processes. Similarly, KEGG enrichment analysis results revealed that IL-8 was related to immune-related biological processes and that it was enriched in multiple cancer-related pathways, such as cytokine–cytokine receptor interaction, IL-17 signaling pathway, chemokine signaling pathway, TNF signaling pathway, NF-kappa B signaling pathway, and chemical carcinogenesis-DNA adducts (Figure 4C).

To further evaluate the potential molecular mechanisms linking IL-8 and BRCA, we conducted GSEA analysis. In the GO term, the top 20 signaling pathways affected by IL-8 are illustrated in Figures S1 and S2. Notably, the high expression of IL-8 was mainly enriched in cancer-related pathways in KEGG terms, which included chronic myeloid leukemia, colorectal cancer, JAK–STAT-signaling-pathway, pancreatic-cancer, small-cell lung cancer, Toll-like receptor signaling pathway, renal cell carcinoma, and acute myeloid leukemia (Figure 4D).

3.4 | Association of the IL-8 expression with TME and immunotherapy response

It is known that immune-infiltrating cells are closely involved in tumor progression and affect the prognosis of cancer patients. Therefore, we explored the association of the IL-8 expression with TME. We noted a significant positive correlation between IL-8 expression and the stromal score, immune score, and estimate score (Figure 5A). Moreover, IL-8 expression was positively correlated with macrophage M0 and mast-activated cells and negatively correlated with mast resting cells and CD8+ T cells (Figure 5B). Further analysis revealed significant differences in IL-8 expression among plasma cells, CD4 memory-activated T cells, regulatory T cells, NK dormant cells, NK-activated cells, monocytes, and neutrophils (Figure 5C). The above results suggest that IL-8 may play a specific role in promoting immune cell infiltration in BRCA.

Immune checkpoints have a major influence on immune cell infiltration and immunotherapy. In order to further determine the role of IL-8 in immunotherapy, we analyzed the correlation between the IL-8 expression and immune checkpoint genes. Figure 5D showed that IL-8 was positively correlated with the



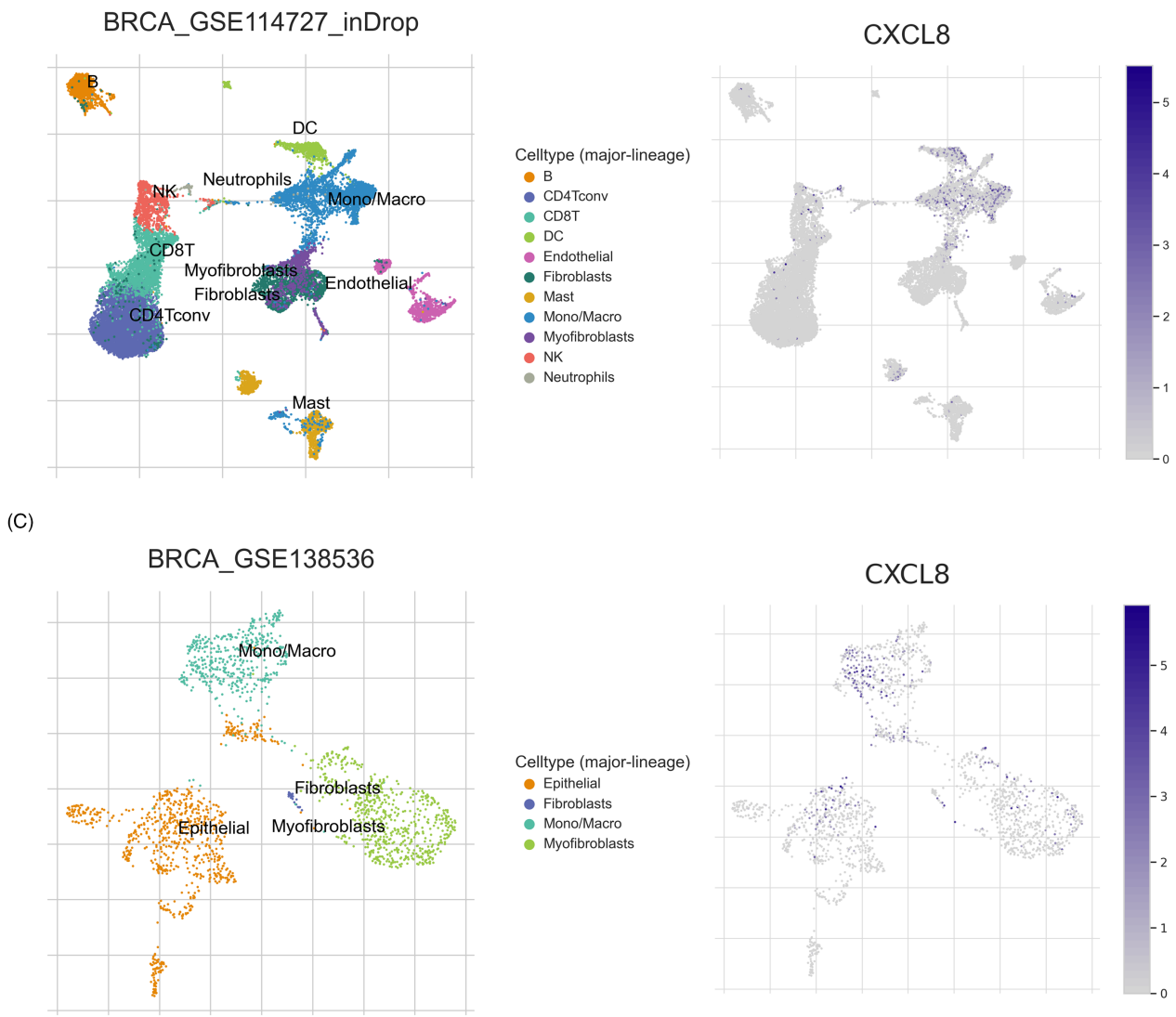


FIGURE 1 Expression of IL-8 in BRCA. (A) IL-8 expression in the TME based on major lineage and malignancy. (B, C) The distribution of different cell types and IL-8 expression in the BRCA_GSE114727_inDrop and BRCA _GSE138536 datasets.

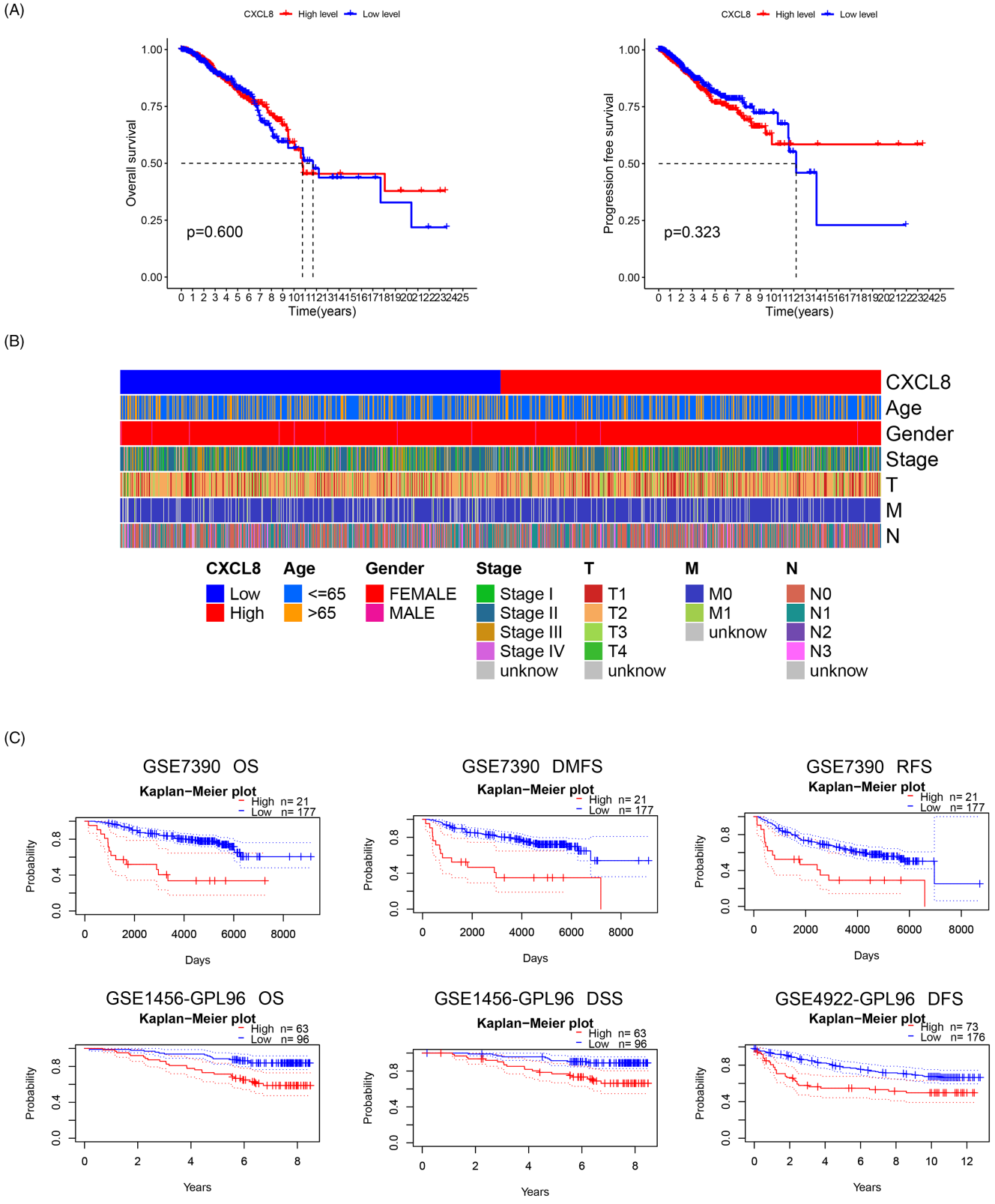


FIGURE 2 Clinical signature of IL-8. (A) The relationship between IL-8 and OS and progression-free survival (PFS). (B) The relationship between IL-8 and clinical features. (C) Survival curves showing the OS, DMFS, RFS, DSS, and DFS in three patient cohorts GSE7390 (n = 198), GSE1456-GPL96 (n = 159) and GSE 4922-GPL96 (n = 249) from the PrognoScan database.

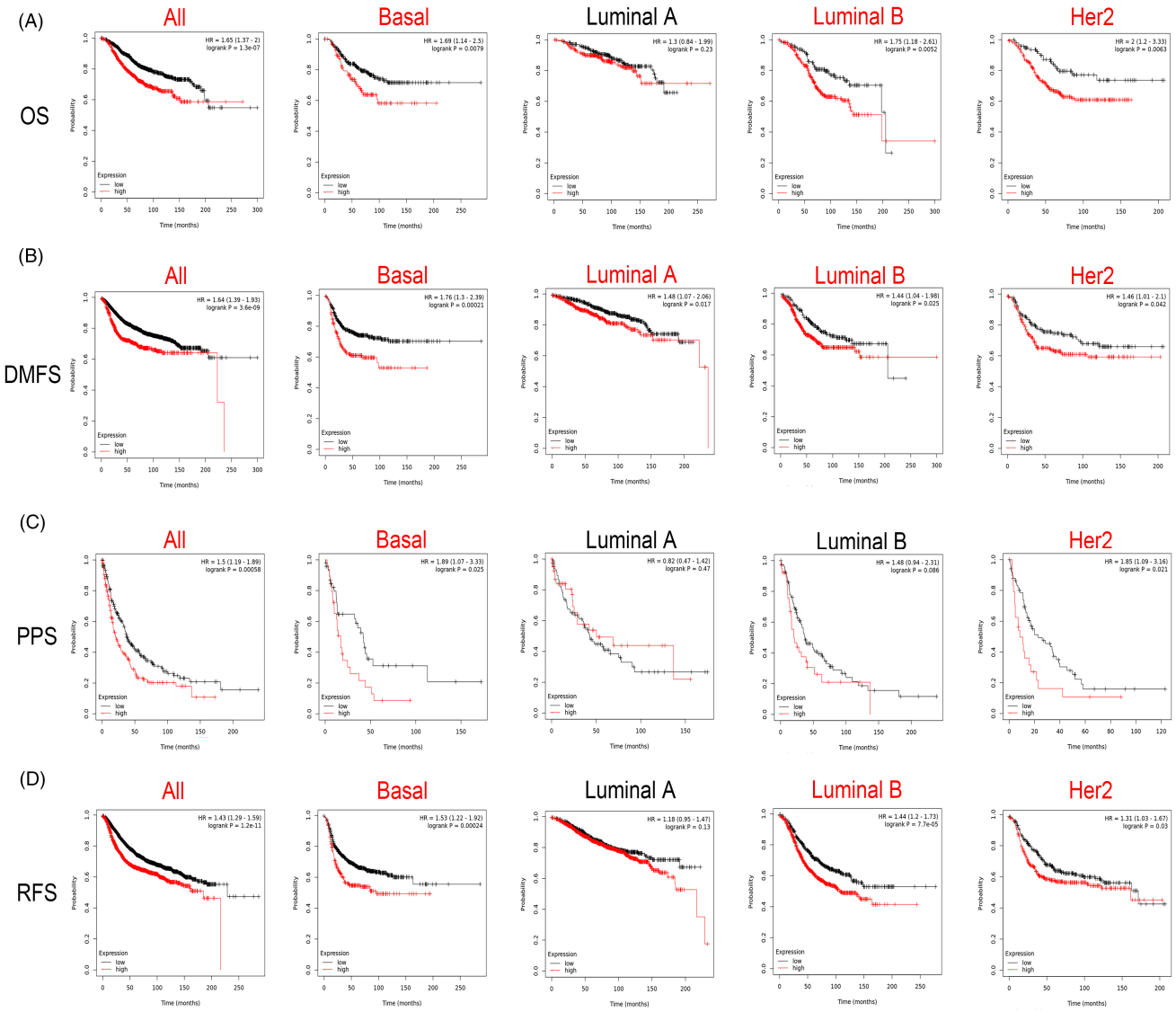


FIGURE 3 Prognostic potential of IL-8 in different subtypes of BRCA. (A–D) The prognostic potential of IL-8 in predicting OS, DMFS, PPS and RFS from the Kaplan Meier Plotter Database.

expression of most immune checkpoint genes. Moreover, we found that the IL-8 expression was positively related to the tumor mutational burden (TMB; Figure 5E). This observation suggested that IL-8 was involved in tumor immune response and hence could be a potential immunotherapy target. To prove this point, PD1 and CTLA4 were recruited for Immune cell Proportion Score (IPS) analyses. The results showed that the IL-8 expression was significantly different in ips_ctla4_neg_pd1_neg (CTLA4 negative response and PD1 negative response), ips_ctla4_neg_pd1_pos (CTLA4 negative response and PD1 positive response), and ips_ctla4_pos_pd1_neg, except for ips_ctla4_pos_pd1_pos (Figure 5F). High IL-8 expression might indicate a poor immunotherapeutic outcome. In summary, these results indicate that IL-8 plays an important role in the tumor immune microenvironment and can predict the immunotherapy response to a certain extent.

3.5 | IL-8 induces EMT

It has been reported that IL-8 contributes to EMT in various types of tumors.[28–30] To elucidate the relationship between IL-8 and EMT, we investigated the correlation between IL-8 and 12 of the EMT-related genes using TIMER. The results showed that except for E-cadherin, there was a significant positive correlation between IL-8 expression and 11 EMT-related genes, including *Fibronectin*, *MMP2*, *N-cadherin*, *Snail*, *Slug*, *SPARC*, *TWIST1*, *TWIST2*, *Vimentin*, *ZEB1*, *ZEB2* (Figure 6A), which suggested that IL-8 could be associated with EMT.

To further verify the relationship between IL-8 and EMT, we performed Western blotting in the BRCA cell line. After the different protein bands were identified, it was found that the Vimentin and N-Cadherin expression were significantly elevated, while the expression of E-Cadherin was significantly downregulated after IL-8 treatment

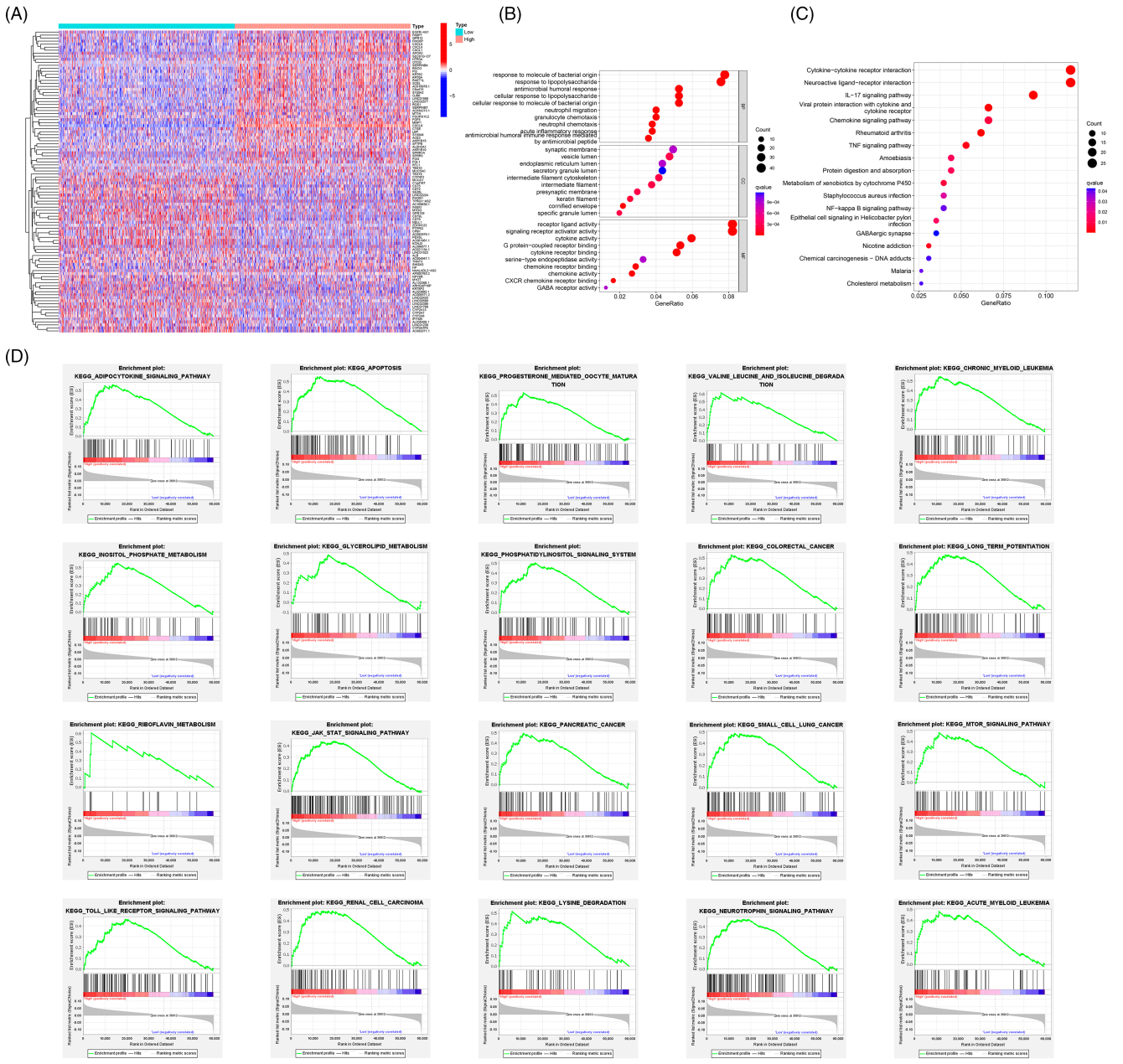


FIGURE 4 Biological function of IL-8. (A) The first 50 genes associated with high and low IL-8 expression, respectively. (B, C) GO and KEGG enrichment analysis for IL-8 analyses. (D) GSEA for samples with high IL-8 expression.

(Figure 6B). Meanwhile, findings from the wound healing and invasion assays revealed that IL-8 enhanced the migration and invasion of breast tumor cells (Figure 6C,D). Chemosensitivity is an important hallmark of EMT, and we found that IL-8 increased the chemoresistance of BRCA cells to multiple chemotherapeutic drugs (Figure 6E,F). Taken together, the above findings suggested that IL-8 promoted EMT.

4 | DISCUSSION

There is increasing evidence that indicates the role of IL-8 in tumor progression. However, the expression signature and prognostic value of IL-8 in BRCA remain unclear. Therefore, understanding the role of IL-8 in BRCA and its related molecular mechanisms would provide new insights for improving the therapeutic efficacy of currently available chemotherapies and immunotherapies. Here, we systematically analyzed the potential functions of IL-8 and confirmed that IL-8 promoted tumor progression by inducing EMT. These findings suggest that IL-8 contributes to the malignant progression of BRCA, which is consistent with its previously reported effects on other malignant tumors. Collectively, we provide new insights into the function of IL-8 and reveal that IL-8 may be a potential therapeutic target for BRCA.

Tumor-infiltrating immune cells affect tumor progression and therapeutic outcomes by regulating immunosuppression and immune escape.[31,32] In BRCA, the crosstalk between

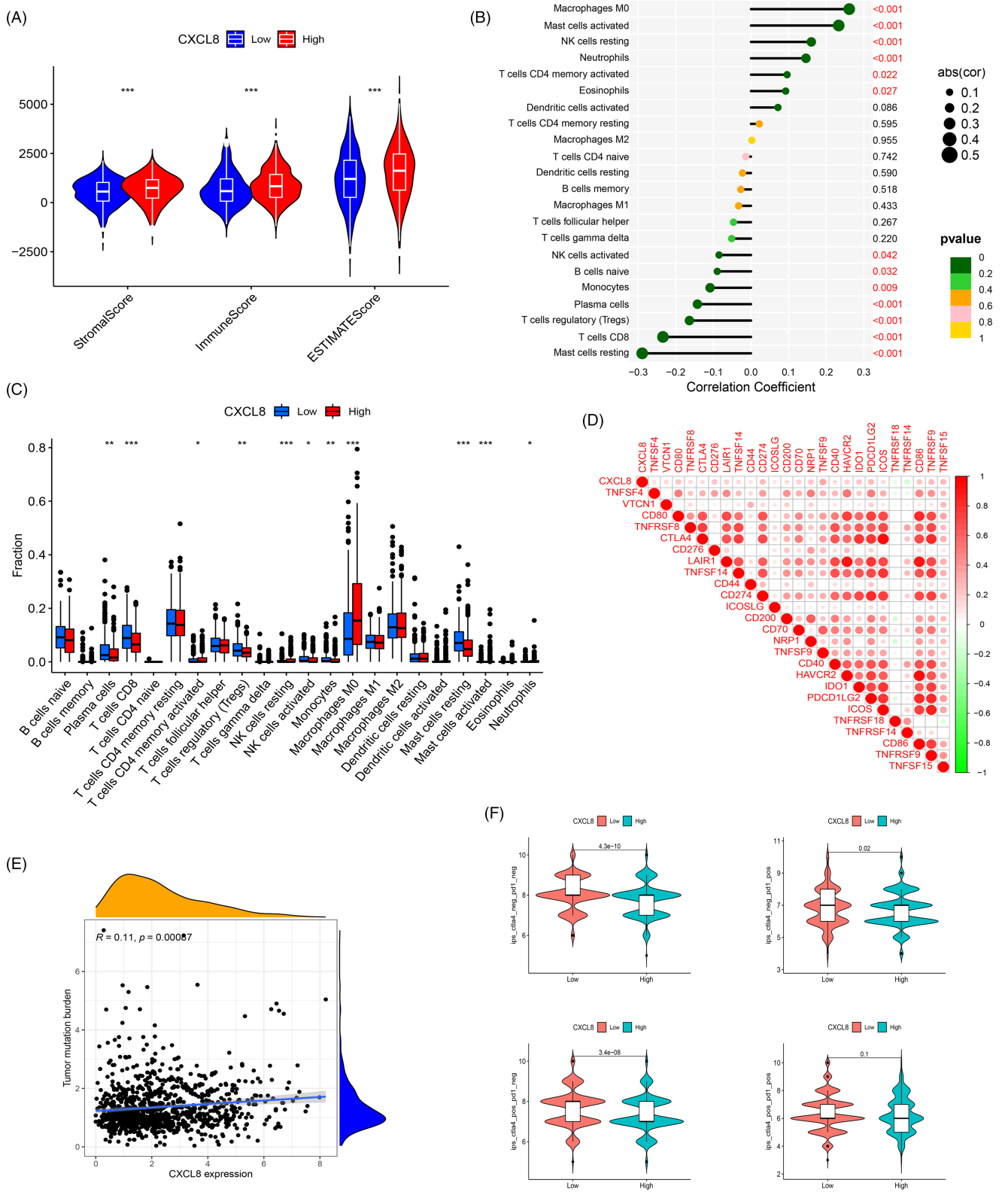


FIGURE 5 The immune characteristics associated with the IL-8 expression in BRCA. (A) IL-8 expression correlated with the stromal score, immune score, and estimated score. (B) IL-8 expression correlated with tumor-infiltrating immune cells. (C) Differential expression of IL-8 in immune cells. (D) Correlation between the IL-8 expression and immune checkpoint genes. (E) The IL-8 expression correlated with TMB. (F) Correlation between the IL-8 expression and IPS scores.

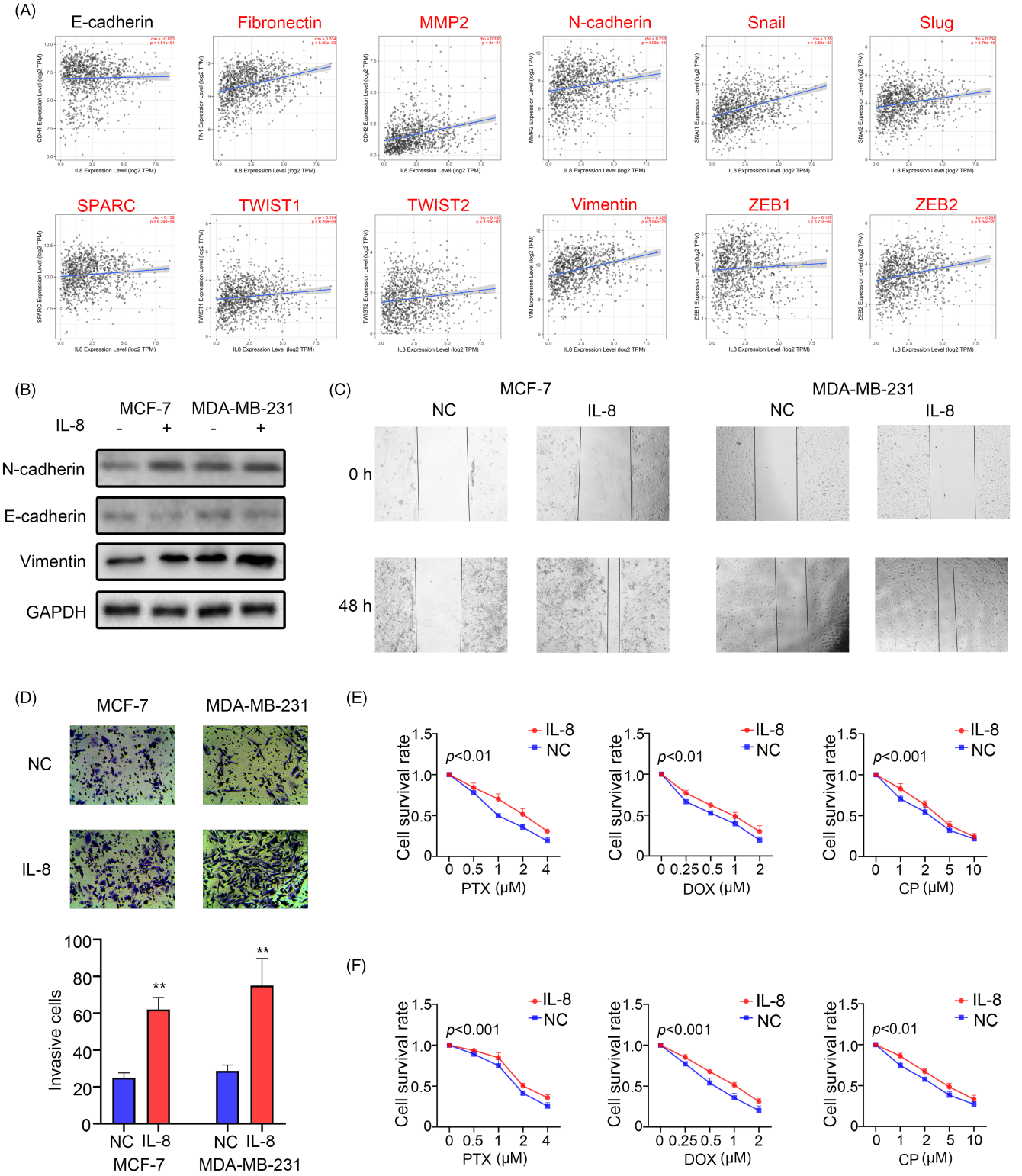


FIGURE 6 IL-8 promotes EMT in BRCA. (A) Correlation between IL-8 and 12 EMT-related genes. (B) Western blot showing the Vimentin, N-Cadherin, and E-Cadherin expression upon treating breast cancer cells with IL-8. (C, D) IL-8 enhanced the wound healing (C) and invasion (D) abilities of breast tumor cells (**$p < 0.01$ vs NC). (E, F) IL-8 enhanced the chemoresistance of MCF-7 (E) and MDA-MB-231(F) cells in vitro (PTX: Paclitaxel, DOX: Doxorubicin, CP: Carboplatin).

tumor-infiltrating immune cells and malignant cells affect tumor classification, recurrence, drug resistance and prognosis, and best reflects the strength and characteristics of tumor-specific immune responses.[33–35] Previous studies have shown that tumor immune infiltrating cells and TMB can predict the response to immunotherapy.[36,37] Higher TMB in tumors is associated with lasting

clinical benefits and hence an attractive target for tumor immunotherapy.[38] Here, we identified that the IL-8 expression was closely associated with most of the tumor-infiltrating immune cells, suggesting that IL-8 may affect tumor development and prognosis by regulating immune cells infiltration within the TME. Furthermore, the TMB levels and IPS score were correlated with the IL-8 expression, which indicated that IL-8 had a certain predictive value in tumor immunotherapy. Therefore, further understanding of the mechanism by which IL-8 regulates tumor immunity may provide novel strategies for cancer immunotherapy.

It has been reported that EMT contributes to tumor invasion, metastasis and treatment resistance,[39] and distant metastasis is known to be the leading cause of death in advanced BRCA patients. EMT is a complex dynamic process that usually involves the downregulation of E-Cadherin and the overexpression of Vimentin. Downregulation of E-Cadherin promotes the production of N-Cadherin and leads to a decrease in intercellular adhesion, resulting in enhanced invasive capacity of the tumors. Vimentin maintains the mesenchymal phenotype of the cells and is positively associated with the invasive and metastatic capacity of the cells. The Snail family of proteins, which includes Snail and Slug, initiates EMT by competitively binding to the promoter sequence of E-Cadherin to repress its transcription.[40] Using the TIMER database, we found that several EMT markers were significantly positively correlated with IL-8 expression, and Western blotting results showed that treating BRCA cells with IL-8 promoted an increase in the protein expression of Vimentin and N-cadherin, and reduced the expression of E-cadherin. Meanwhile, we demonstrated that IL-8 not only enhanced the ability of tumor cells to migrate and invade, but also enhanced their resistance to various chemotherapeutic drugs in vitro. These results indicated that IL-8 could promote tumor progression by regulating EMT, and might serve as a new therapeutic target for modulating EMT in BRCA.

With the deepening of research, the relationship between EMT and tumor immune response regulation is considered to be interactive. On one hand, the cells of the immune system, such as macrophages, cancer-related fibroblasts, NK, and Tregs induce EMT in tumor cells by secreting cytokines and chemokines.[41,42] On the other hand, tumor cells producing EMT act on the immune system through immunosuppression and escape.[43,44] In addition, EMT may affect the infiltration of immune cells. Specifically, some cytokines involved during EMT, such as IFN-γ, TGF-β, and TNF-α may cause TME modification.[45,46] Moreover, tumor cells undergoing EMT may generate an inflammatory environment by releasing soluble factors to recruit immune cells, which may assist in tumor growth. According to our data, IL-8 may play a special role in the tumor immune microenvironment, similar to these factors, although the specific relationship and mechanism remain unclear. However, inflammation is the key inducing factor of EMT in the process of cancer progression. As an inflammatory mediator, the molecular mechanism through which IL-8 promotes EMT and influences anti-tumor immunity deserves further investigation.

Although we found that IL-8 expression correlated with immune cell infiltration and immune response in BRCA through public databases, the corresponding in vitro experimental validation and animal models were lacking. Therefore, the mechanism by which IL-8 modulates immune responses and EMT in BRCA requires further validation in vitro and in vivo.

5 | CONCLUSION

Our study provides some insight into the specific role and potential function of IL-8 in modulating the immune signature and EMT in BRCA. In summary, our findings suggest IL-8 as a predictive biomarker for the prognosis and immunotherapy outcomes in BRCA patients, and that it promotes tumor progression by inducing EMT.

AUTHOR CONTRIBUTIONS

Huifeng Liao and Huayan Li conceived the idea and performed the research; Jin Song and Junhua Dong analyzed the data; Hongye Chen and Huiyan Si participated in the design and provided some reagents; Xue Bai and Jiandong Wang conducted the study and provided funds. All authors read and approved the final manuscript.

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FUNDING INFORMATION

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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